VARIATION IN DICOT C_4 TYPE LEAF ANATOMY IN THE HUNGARIAN FLORA

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Abstract

A considerable variation in Kranz anatomy was encountered in the Hungarian angiosperm flora, we utilised the examination results of in total 298 species. The anatomical structure of the leaves has been examined by using light microscope. Altogether we found 23 C_4 dicot species in the *Amaranthaceae* (10), *Chenopodiaceae* (8), *Euphorbiaceae* (3), *Portulacaceae* (1) and *Zygophyllaceae* (1) families. It is demonstrated that there are possibly two different types of Kranz anatomy. While in the typical Kranz syndrome each vascular bundle is surrounded by a bundle sheath, these non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf. The *Chenopodiaceae* showed the greatest variation with three structural types for eight species, and with the most unusual leaf structural variants (i.e. kochioid and salsoloid types). The non-typical Kranz structures mainly occur among plants of the most extreme habitats (semidesert-like saline and sand grasslands). The observed anatomical variation reflects the poliphyletic origin of the C_4 photosynthesis and the diversity of biotopes inhabited by C_4 plants.

Key words: Amaranthoid type, Atriplicoid type, Euphorboid type, Kochioid type, non-typical Kranz anatomy, Salsoloid type, typical Kranz anatomy

Introduction

Since its discovery in the mid-sixtes, the C_4 photosynthetic pathway has been continuously staying in the centre of scientific attention. Examining corn leaves HABERLANDT (1918) recognized a sheath of vast, thin-walled cells around vascular bundles, which was surrounded by radially organised palisade-like mesophyll cells. He termed this leaf structure Kranz anatomy. During further physiological studies

HATCH (1976, 1987) discovered that the C_4 pathway of photosynthesis is associated with this structure.

C_4 plants occur in more than 200 genera of 18 angiosperm families, ca. 1000 species (DOWNTON, 1975; WATSON and DALLWITZ, 1993) fix carbon via the C_4 pathway (HATTERSLEY, 1987; RAGHAVENDRA and DAS, 1978). During this work a remarkable variation in the anatomical structure had been observed and classifications of Kranz anatomy types was elaborated (e.g. CAROLIN et al., 1975; HATTERSLEY and WATSON, 1976). Nevertheless, all anatomical variants share one fundamental feature (DENGLER et al., 1985); i.e. the leaf chlorenchyma is always differentiated into two functional units: the mesophyll and the bundle sheath. By this arrangement the two major steps of C_4 metabolism becomes spatially separated within the leaf. Namely, CO_2 is fixed into malate or aspartate in the mesophyll (primary carbon assimilation /PCA/ tissue), then the acids are transported into the bundle sheath (photosynthetic carbon reduction /PCR/ tissue). Here they are C_4 acid decarboxilation and the resulting CO_2 enters the Calvin cycle and finally starch is formed. Further research revealed biochemical subtypes of the C_4 metabolism (see HATCH, 1987 for review), and relationships have been found between leaf anatomy and biochemical subtypes (DOWNTON, 1975; DOWNTON et al., 1969; EDWARDS and HUBER, 1981; GAMALEJ and VOZNYESZENSZKAJA, 1986; GUTIERREZ et al., 1974). According to the primary product of CO_2 fixation, the decarboxylation enzyme and the anatomy and ultrastructure of bundle sheath cells in the case of dicot plants the following C_4 subtypes have been distinguished (GUTIERREZ et al., 1974; HATTERSLEY, 1984; HATCH, 1987; VOZNYESZENSZKAJA and GAMALEJ, 1986).

1. NADP-ME. The first product of CO_2 fixation is malate, that is decarboxylated in the bundle sheath by NADP malic enzyme (NADP-ME). Bundle sheath chloroplasts lack grana and are centripetally arranged. Mesophyll chloroplasts contain grana.
2. NAD-ME. Carbon dioxide is fixed into aspartate, the decarboxylating enzyme is NAD malic enzyme (NAD-ME). Bundle sheath chloroplasts are granal and centripetally positioned, while mesophyll chloroplasts lack grana.

The Kranz anatomy has been studied particularly in detail in the *Chenopodiaceae* (CAROLIN et al., 1975; DOWNTON et al., 1969; DENGLER et al., 1995; KNAPP-ZINN, 1984; LIU and DENGLER, 1994; PATRIGNANI et al., 1993; PJANKOV and VAHRUZSEVA, 1989; OSMOND et al., 1980; VASZILEVSZKAJA and BUTNIK, 1981; WELKIE and CALDWELL, 1970).

The screening of the Hungarian angiosperm flora for the occurrence of C_4 plants has been completed recently (KALAPOS, 1991; KALAPOS et al., 1997; NYAKAS, 1991, 1992; NYAKAS and KALAPOS, 1996). During this work leaf anatomy was one of the traits used for the determination of the photosynthetic pathway type of plants. This paper summarizes the variation of Kranz anatomy in dicot plants encountered during the survey.

Table 1. Dicot C_4-species in Hungarian Angiosperm flora.
Where literature data are used reference to source is given in superscript. Life forms: Th = therophytes, H = hemicryptophytes, Ch = chamaephytes, N = nanophanerophytes, (Phytogeographical and life forms classification of species follows of SIMON 1992). Literature sources: 1. MATEU 1993, 2. WELKIE et al. 1970

Anatomical structure	Taxon	Floristic element	Life forms
Typical Kranz anatomy			
Amaranthoid type			
	Amaranthaceae		
[1,2]Amaranthus albus L.		adventive	Th
A. blitoides S. Watson		adventive	Th
[1,2]A. bouchonii Thell.		adventive	Th
A. chlorostachys Willd.		cosmopolitan	Th
[1,2]A. crispus (Lesp. et Théven) N. Terrac		adventive	Th
A. deflexus L.		adventive	H
[1,2]A. graecizans L.		south-eurasian	Th
[1,2]A. lividus L.		cosmopolitan	Th
A. patulus Bert.		adventive	Th
A. retroflexus L.		cosmopolitan	Th
	Zygophyllaceae		
Tribulus terrestris L.		cosmopolitan	Th
Euphorboid type			
	Euphorbiaceae		
Euphorbia humifusa Willd.		adventive	Th
E. maculata L.		adventive	Th
E. nutans Lag.		adventive	Th
	Portulacaceae		
Portulaca oleracea L.		cosmopolitan	Th
Atriplicoid type			
	Chenopodiaceae		
Atriplex rosea L.		south-eurasian	Th
A. tatarica L.		eurasian-(med)	Th
Non typical Kranz anatomy			
Kochioid type			
Kochia laniflora (Gmel.) Borb.		eurasian	Th
K. prostrata (L.) Schrad.		eurasian-(med)	Ch-N
K. scoparia (L.) Schrad.		eurasian	Th
Salsoloid type			Th
Salsola kali L.		eurasian-(med)	Th
S. soda L.		eurasian-(med)	Th
Camphorosma annua Pall.		ponto-pannonic	

Materials and Methods

C_4 plants occur in more than 200 genera of 18 angiosperm families (15 families in dicot and 3 in monocot), ca. 1000 species (DOWNTON, 1975; WATSON and DALLWITZ, 1993) fix carbon via the C_4 pathway. In search for dicot C_4 plants in Hungarian flora 66 species representing 15 families have been examined. We used additional literature data, if available, with the source always indicated. In our test, mostly those families were screened where the occurence of C_4 species had reported earlier (CAROLIN et al., 1975; DENGLER et al., 1995; LIU and DENGLER, 1994; MATEU, 1993). Nomenclature follows DAHLGREN et al. (1985) and SIMON (1992).

The leaf anatomy was studied on cross section of leaf blades (mature stem leaves) and wherever possible fresh plant material was used to prepare sections for examination with the light microscope. This material fixed with in a 1:1:1 mixture of abs. alcohol, glycerine and distilled water. Sections were cut 10–12 μm thick and the samples were either stained with azur-hematoxilin or treated with the lugol solution to identify the localisation of starch accumulation within the chlorenchyma. Light microscopic pictures of various enlargement were recorded by using a computer image processing system and stored on CD-ROM.

Results and Discussion

Altogether we found wild 23 C_4 dicot species in the *Amaranthaceae* (10), *Chenopodiaceae* (8), *Euphorbiaceae* (3), *Portulacaceae* (1) and *Zygophyllaceae* (1) families (Table 1.). These species in Hungary are mainly cosmopolitan plants, or naturalised aliens or weeds, only few of them are native. They are predominantly annual species inhabit dry grasslands, saline areas, temporally exposed riverbeds and disturbed sites (COLLINS and JONES, 1985; KALAPOS, 1991; KALAPOS et al., 1997; NYAKAS, 1992). Hungary's geographical situation and the diversity of its natural habitats can explain that its flora is richer in C_4 species than of other temperate — zone countries.

The leaf anatomy of dicot C_4 plants is more diversified than of the monocot ones. In addition to the so-called typical Kranz anatomy (Kranz-rosette mesophyll, HABERLANDT, 1918) characteristic to grasses, several non-typical arrangements occur. While in the typical Kranz syndrome each vascular bundle is surrounded by a bundle sheath, these non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf, which is surrounded by the PCA mesophyll tissue. Leaf shape (cylindrical or semi-cylindrical) appears to have no effect on the basic anatomical structure within each type.

Typical Kranz anatomy

The leaf blade is flat, dorsiventral with amphistomatic epidermis, the adaxial and abaxial sides are clearly distinguishable. The Kranz rosettes are similar to those in grasses, though here they develop around ramified leaf veins. However the bundle sheath is always single layered. Vast, mostly centripetally arranged chloroplasts fill the parenchymatous bundle-sheath cells (Fig. 1). The following variation had been observed among the studied species.

Amaranthoid type (Fig. 1/a)
The bundle-sheath is complete, the mesophyll is isopalisade. It is characteristic to *Amaranthus* and *Tribulus* species.

Euphorboid type (Fig. 1/b)
The bundle-sheath is complete, the radially arranged PCA tissue is palisade only on the adaxial side. Abaxial side cells are less elongated in form (overlaying a spongy mesophyll), as they adjoin the cells of the water-holding ground tissue. It is characteristic to *Euphorbia* and *Portulacca* species. In the case of *Euphorbia humifusa* scattered Kranz cells can also be seen between vascular bundles.

Atriplicoid type (CAROLIN et al., 1975, Fig. 1/c)
The Kranz cells form parenchymatous sheath around the vascular bundles, except for the gap beside the phloem, with a radial arrangement of the PCA tissue. The mesophyll by which is palisade only on the adaxial side, while its cells on the abaxial side are less elongated.

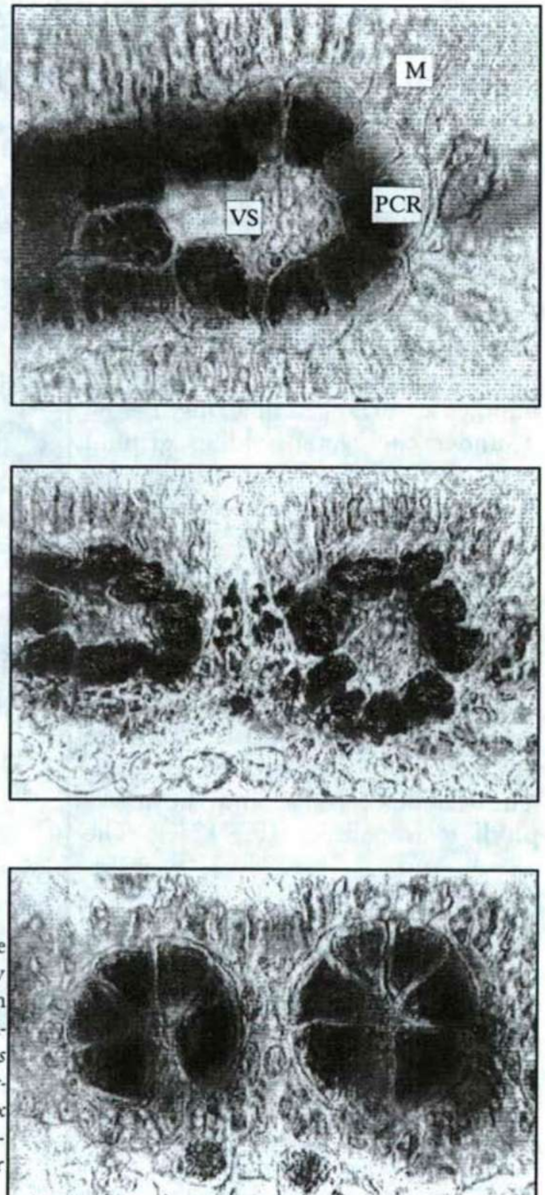

Figure 1. Micrographs of portion of leaf blade cross sections with typical Kranz anatomy (dark areas indicate the place of starch accumulation within the leaf [lugol staining]). a: Amaranthoid type — *Amaranthus retroflexus*, b: Euphorboid type — *Euphorbia humifusa*, c: Atriplicoid type — *Atriplex rosea* (x 540). Abbreviations: PCR = parenchymatous bundle sheath, VB = vascular bundle, M = mesophyll.

Non typical Kranz anatomy

Non typical types contain a „collective" Kranz sheath organised around multiple bundles in the leaf. The non-typical Kranz structures mainly occur among plants of the most extreme habitats (semidesert-like saline and sand grasslands).

Kochioid type (Fig. 2)
The leaf blade is cylindrical or flat, the epidermis is amphistomatic. The Kranz-cells (PCR tissue) form arcs along the xylem of peripheral bundles. If the leaf blade is cylindrical (*Kochia laniflora, K. prostrata*) the bundles are arranged in a ring. Below it under the water-holding ground tissue (hypodermis) the palisade parenchyma (PCA tissue) forms a continuous layer (Fig. 2/a). Mostly the leaves of this type have extensive central aqueous tissue. *Kochia scoparia* represents a special case of this type (CAROLIN et al., 1975) in which the central aqueous tissue has been suppressed and the vascular bundles opposite each other pressed together. The leaf blade is flat and the mesophyll is isopalisade (Fig. 2/c). The

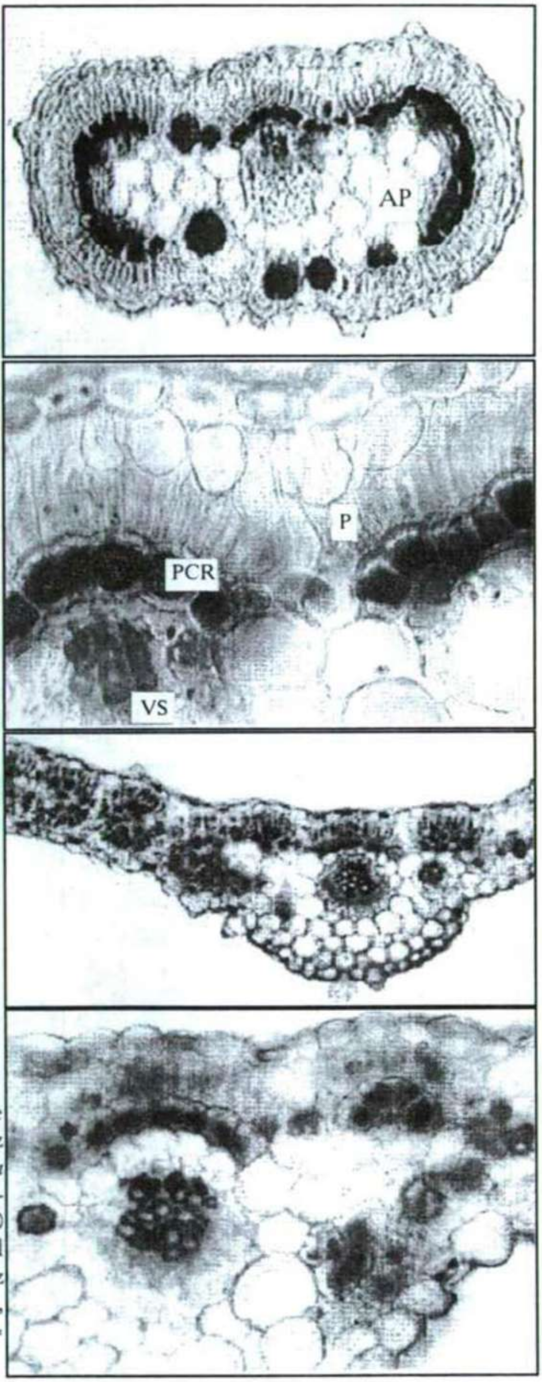

Figure 2. Micrographs of portion of leaf blade cross sections with non-typical Kranz anatomy. Kochioid type: a. and b. *Kochia laniflora* (a: x 170, b: x 540 [lugol staining]), c. and d. *Kochia scoparia* (c: x 170 [azur-hematoxylin staining], d: x 270 [lugol staining]). Abbreviations: PCR = Kranz cells (form arcs along beside the xylem), VB = vascular bundle, P = palisade, AP = aqueous tissue.

Kranz cells thus appear as a partial bundle sheath, interrupted laterally, around centric bundles towards the outer parts of the leaf transection whilst towards the centre of the leaf the aqueous tissue still separates opposite bundles and the Kranz cells appear as in other Kochioid types.

Salsoloid type (Fig. 3)
The leaf blade is cylindrical. Below the epidermis the palisade parenchyma (PCA tissue) forms a continuous layer. With this the Kranz cells (PCR tissue) connects, which also makes an unbroken sheath (Fig. 3/a and 3/b). Within this small bundles are situated in a ring. This leaf anatomy is particularly by interesting, most of the Kranz cells have no direct connection with the vascular bundles, as they adjoin the cells of the water-holding ground tissue. In the center of the cylindrical leaf blade a large vascular bundle is situated without Kranz tissue (PATRIGNANI et al., 1993). Besides the *Salsola* species this structure is characteristic to *Camphorosma annua* too (Fig. 3/c).

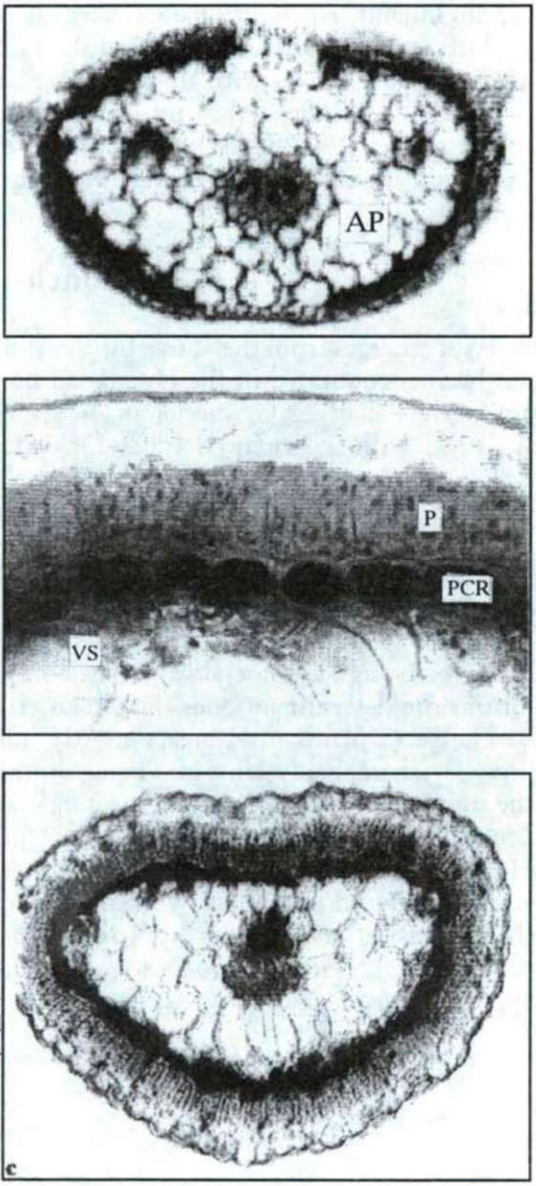

Figure 3. Micrographs of portion of leaf blade cross sections with non-typical Kranz anatomy [lugol staining]. Salsoloid type: a. and b. *Salsola kali* (a: x 50, b: x 540), c. *Camphorosma annua* (x 50). Abbreviations: PCR = „collective" Kranz sheath, VB = vascular bundle, P = palisade, AP = aqueous tissue.

In the Hungarian flora there are genuses (*Atriplex, Euphorbia*) in which either C_3 or C_4 species can be found. So far only C_3 (*Heliotropium, Sueda, Salicornia*) or C_4 (*Kochia*) species were recorded. However according to literature data (CAROLIN et al., 1975; VASZILEVSZKAJA and BUTNIK, 1981) in these genuses both C_3 and C_4 species inhabit. An additional so-called suedoid type (named after the genus *Sueda*) C_4 leaf anatomy had been distinguished in the *Chenopodiaceae* (CAROLIN et al., 1975), but no species with this structure occurs in the Hungarian flora (NYAKAS and KALAPOS, 1996). Also, no ventro-palisade structure characteristic to C_4 *Salicornia* species (VASZILEVSZKAJA and BUTNIK, 1981) was observed, as only one C_3 *Salicornia* species (*S. europaea*) grows in this country.

Conclusions

As it emerges from the above list a considerable diversity of Kranz leaf anatomy has been encountered in the Hungarian flora, since eight structural variants occur among less than 23 C_4 species in dicot. The *Chenopodiaceae* shows the greatest variation with 3 structural types for eight species, and with the most unusual Kranz anatomy variants (kochioid and salsoloid types). This high anatomical diversity also reflects the poliphyletic origin of the C_4 photosynthesis discussed in detail elsewhere (e.g. MONSON, 1989; EHLERINGER and MONSON, 1993) and the high diversity of biotopes inhabited by C_4 plants. The non-typical Kranz structures mainly occur among species of the most extreme environments (semidesert-like saline and sand grasslands). This fact shows that in forming of different photosynthetic pathways are not always the family relationships but the ecological conditions the determinant ones (KALAPOS et al., 1997).

Native C_4 plants in Hungary mostly inhabit dry grasslands or salt affected habitats, wich probably came into being during 8000–5000 years before present, when the steppe vegetation of the Great Hungarian Plain started to develop (JÁRAI-KOMLÓDI, 1987; ZÓLYOMI and FEKETE, 1994). However, noumerus C_4 species in the Hungarian flora are naturalised aliens (e.g. *Amaranthus* spp., *Euphorbia* spp.) which were introduced in the last two centuries. Human activities greatly contributed to their invasion by creating appropriate habitats for them (ruderal sites, waste ground etc.). The invasion is continuing even today, as shown by recent appearance of a new C_4 weed, *Amaranthus bouchonii* (SOLYMOSI and PRISZTER, 1984) in Hungary.

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